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Genotype distribution of *Mycobacterium tuberculosis* in the Aegean Region and associated demographic factors

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Background/aim: The Aegean Region is the second-ranking region in Turkey according to the Human Development Index and population density and it hosts 1/8 of Turkey's population. İzmir is the largest city of the region, receiving internal migration both from inside and outside the region. The tuberculosis incidence in İzmir is lower than overall in Turkey: 17.7/100,000 in 2011. Our aims were to determine genotypes of *Mycobacterium tuberculosis* isolates; to explore possible associations between genotypes with case-demographic data, clinical presentation, and antimicrobial susceptibility patterns; and to determine variations in genotype distribution of strains isolated in Ege University Hospital, İzmir.

Materials and methods: Forty-nine *M. tuberculosis* isolates from 49 patients in 1996–2000 and 421 *M. tuberculosis* isolates from 421 patients in 2009–2014 were spoligotyped. Drug susceptibility testing and demographic data of the 421 isolates were investigated. Chi-square, Student's t, and Mann–Whitney U tests were used for analyses.

Results: Among the 470 *M. tuberculosis* strains, 132 different spoligopatterns were identified and 46 different clusters for 384 strains were determined. The most predominant spoligotypes were ST53 (n = 116; 24.7%) and ST41 (n = 38; 8.1%), followed by ST50 (5.7%), ST284 (4.7%), and ST4 (4.3%), respectively. ST53 was the most predominant type in both sexes. Multidrug resistance (MDR) was determined in 12 isolates, of which six were ST1.

Conclusion: As a consequence of worldwide migration and increasing status of HIV-infected hosts, the increasing prevalence of Beijing strains with higher MDR rates may threaten disease control programs. With its increasing trend, ST284 could replace ST41 in the following years in this region.

Key words: Spoligotyping, *Mycobacterium tuberculosis*, genetic diversity, tuberculosis epidemiology

1. Introduction

According to World Health Organization data, although there is a decreasing trend in tuberculosis (TB) incidence, prevalence, and mortality worldwide, the number of new TB cases in 2014 was estimated as 9.6 million, including 5.4 million men, 3.2 million women, and 1 million children. In 2014, 1.5 million individuals died because of TB (1).

The incidence of TB in Turkey has dropped from 52.2/100,000 in 1980 to 26/100,000 in 2005 and 18/100,000 in 2014 (1–3). In total, 15,679 new TB cases, consisting of 59% pulmonary, 37% extrapulmonary, and 4% simultaneous pulmonary and extrapulmonary TB cases, were reported in Turkey in 2011. Among these cases, 59% of patients were males and 41% were females, and the highest incidence rates for both sexes were over the age of 65 years (2).

The Aegean Region is the second-ranking region in Turkey according to the Human Development Index and population density and it hosts 1/8 of Turkey's population. İzmir is the largest city of the region and Turkey's third largest city with its 4,113,000 inhabitants receiving internal migration both from inside and outside the region (4,5). TB incidence in İzmir is lower than overall in Turkey: 17.7/100,000 in 2011. In İzmir, a total of 700 cases were reported in 2011 and 61% of the cases were pulmonary, 33% extrapulmonary, and 6% both pulmonary and extrapulmonary. Among the patients, 64% were male and 36% were female (2).

The aims of this study were to determine the genotypes of *Mycobacterium tuberculosis* strains isolated in Ege University Medical School's hospital in 2009–2014; to

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explore the possible associations between genotypes and case-demographic data, clinical presentation, and antimicrobial susceptibility patterns; and to determine variations in genotype distributions of the strains.

2. Materials and methods

2.1. *M. tuberculosis* isolates

All of the *M. tuberculosis* strains, which were isolated by automated liquid culture system in the hospital of the Ege University Medical School between 2009 and 2014, were included in the study. In order to detect a possible shift in the distribution of *M. tuberculosis* strains throughout the years, 49 strains isolated between 1996 and 1998 were also spoligotyped.

The 421 *M. tuberculosis* strains isolated in 2009–2014 constitute 10%, 5%, and 0.5% of all the strains isolated in İzmir, in the Aegean Region, and in Turkey, respectively, in the same period (2). Among the 421 strains studied, 268 (63.9%) were isolated from pulmonary TB and 131 (31.3%) from extrapulmonary TB patients' clinical samples, while 20 (4.8%) patients had both pulmonary and extrapulmonary TB, and the remaining two isolates' locations were undefined.

2.2. Demographic data

Demographic data of the 421 patients in the 2009–2014 period were obtained from the Ege University Medical School's Medical Microbiology Department's Mycobacteriology Laboratory records and the hospital's database. It was not possible to reach these data for the 1996–1998 period so this period was excluded from the demographic analyses.

2.3. Spoligotyping

A standard spoligotyping procedure was applied according to the manufacturer's instructions (Isogen Life Science Spoligotyping Kit, the Netherlands) (6). All spacers in the direct repeat (DR) region were amplified with PCR using DRa (biotin-labeled) and DRb primers. The biotin-labeled amplified spacers were hybridized with different spacer probes that covalently bond to the membrane. The spacers hybridized on the membrane were incubated with HRP-conjugate and substrate. The membrane was treated with X-ray or visualized by Fusion FX7 (Vilber Lourmat, France) chemiluminescence imaging systems to detect hybridized spacers. The presence of a hybridized spacer was considered as a positive signal while the lack of spacer hybridization was considered as a negative signal. The binary spoligotype values obtained for each strain were entered into the database located at http://www.pasteur-guadeloupe.fr/tb/bd_myco.html, and the Spoligo International Type (SIT) number and the family that it belongs to were determined.

2.4. Drug susceptibility testing (DST)

DST was performed during routine laboratory procedures. Susceptibility to rifampicin (RIF), isoniazid (INH), ethambutol, and streptomycin was determined by the agar proportional method or with the MGIT960 automated system (BD, USA) (7).

2.5. Data analysis

Two or more patient isolates with the same spoligopattern were considered as a cluster. Epidemiologic and clinical data of the strains were compared with the chi-square test or Fisher's exact test. The most frequent eight types (total n > 10) were further analyzed according to sex, age, and place of birth by creating dichotomous variables encoded as the specific type of interest versus the total of others, comprising orphans. Trends in consecutive age groups and in study years grouped into four consecutive groups (1996–98, 2009–10, 2011–12, 2013–14) and were tested with chi-square tests for trend analysis. Statistical significance was set at P < 0.05 (CI: 95%).

3. Results

3.1. *M. tuberculosis* spoligotypes and their distribution in different study years

Among the 470 *M. tuberculosis* strains, 132 different spoligopatterns were determined, 384 strains formed 46 clusters, and the clustering rate was 81.7%. The most dominant spoligotype was ST53 (n = 116; 24.7%), followed by ST41 (LAM7-TUR), ST50, ST284, and ST4. Among *M. tuberculosis* strains, 54 of them had no corresponding SIT in the database. The most dominant spoligotype was the T family (n = 219; 46.6%), followed by Haarlem (n = 75; 16%), LAM (n = 58; 12.3%), unknown (n = 30; 6.4%), S (n = 14; 3%), Beijing (n = 11; 2.3%), CAS (n = 4; 0.9%), and X (n = 4; 0.9%) families. There was no *M. tuberculosis* strain belonging to the EAI family. ST41 was found in 18.4% and ST4 in 8.2% of the strains isolated in 1996–1998 while their rates were 6.9% and 3.6%, respectively, in 2009–2014. Conversely, ST284 and ST1 (Beijing) were not detected among the strains isolated in 1996–1998 but they were detected in 5.2% and 2.6% of the strains in 2009–2014 (Table 1).

A significantly decreasing trend was observed for ST41, with rates of 18.4%, 8.7%, 7.4%, and 4.0% isolated in 1996–98, 2009–10, 2011–12, and 2013–14, respectively (P for trend = 0.005). When the 1996–1998 period was excluded from the trend analyses, an increasing trend for ST47 was observed with rates of 1.9%, 4.4%, and 6.5% in 2009–10, 2011–12, and 2013–14, respectively (P for trend = 0.050).

3.2. Demographic characteristics of the patients with *M. tuberculosis* strains isolated

The demographic data of the patients are summarized in Table 2. The ratio of the age group of 15–44 years was

Table 1. The distribution of *M. tuberculosis* strains' spoligotypes according to different study years (n, %).

Clade	SIT number	2009–2014	1996–1998	Total	P*
T1	53	105 (24.9)	11 (22.4)	116 (24.7)	0.702
LAM7-TUR	41	29 (6.9)	9 (18.4)	38 (8.1)	0.011**
H3	50	24 (5.7)	3 (6.1)	27 (5.7)	0.753**
T1	284	22 (5.2)	0	22 (4.7)	0.151**
H1	47	17 (4.0)	2 (4.1)	19 (4.0)	1.000**
Unknown	4	16 (3.8)	4 (8.2)	20 (4.3)	0.144**
Beijing	1	11 (2.6)	0	11 (2.3)	0.615**
T2	52	10 (2.4)	0	10 (2.1)	0.609**
H3	262	9 (2.1)	0	9 (1.9)	0.607**
T1	7	7 (1.7)	0	7 (1.5)	1.000**
S	34	6 (1.4)	1 (2.0)	7 (1.5)	0.540**
T1	196	6 (1.4)	1 (2.0)	7 (1.5)	0.540**
LAM9	42	6 (1.4)	0	6 (1.3)	1.000**
Ambiguous LAM5 LAM4	608	2 (0.5)	2 (4.1)	4 (0.9)	NA
Other types	-	99 (23.5)	14 (28.6)	113 (24.0)	0.433
Orphan	-	52 (12.4)	2 (4.1)	54(11.5)	0.086
Total		421 (100.0)	49 (100.0)	470 (100.0)	

*The results of the comparisons did not change when the orphan group (n = 54) was excluded from the analyses, **Fisher's exact test, NA: not available due to insufficient numbers.

Table 2. The demographic characteristics of patients with *M. tuberculosis* strains isolated in 2009–2014 (n, %).

Characteristic	Sex, n (%)		Total
	Males	Females	
Sex (n = 421)	246 (58.4)	175 (41.6)	421 (100.0)
Age groups (n = 420)			
0–4	0 (0)	1 (0.6)	1 (0.2)
5–14	3 (1.2)	1 (0.6)	4 (1.0)
15–24	20 (8.2)	22 (12.6)	42 (10.0)
25–34	30 (12.2)	29 (16.6)	59 (14.0)
35–44	31 (12.7)	25 (14.3)	56 (13.3)
45–54	37 (15.1)	18 (10.3)	55 (13.1)
55–64	58 (23.7)	29 (16.6)	87 (20.7)
≥65	66 (26.9)	50 (28.6)	116 (27.6)
Birth place (n = 418)			
Marmara Region	26 (10.6)	18 (10.4)	44 (10.5)
Aegean Region	117 (47.8)	95 (54.9)	212 (50.7)
Mediterranean Region	13 (5.3)	5 (2.9)	18 (4.3)
Middle Anatolia	17 (6.9)	14 (8.1)	31 (7.4)
Southeast Anatolia	7 (2.9)	10 (5.8)	17 (4.1)
Black Sea	16 (6.5)	4 (2.3)	20 (4.8)
East Anatolia	33 (13.5)	21 (12.1)	54 (12.9)
Abroad	16 (6.5)	6 (3.5)	22 (5.3)

higher in females, while the ratio of the age group of 45–64 years was higher among males (P = 0.062, Table 2). Among the patients, 49.6% (n = 209) were born outside of the Aegean Region. The mean age of patients with ST53 was significantly higher than patients with other types (Table 3). The remaining seven most common types did not show a significant difference in mean age in comparison to the rest of the patients. However, rare types were isolated from significantly younger patients (Table 3).

3.3. Age groups, sex, and birth place distribution of different spoligotypes

ST53, ST4, and ST47 were seen more frequently in older age groups (≥45 years) while ST41, ST284, and ST1 were more frequent in younger age groups (15–44 years). The most predominant type among both sexes, ST53, was followed by ST284 and ST41 in women and ST41 and ST50 in men (Table 4). Three out of the 11 patients with the Beijing strain isolated were born in the Middle Asian Turkic states, while eight were born in Turkey. When different types were compared according to birth place, grouped as Turkey versus abroad, the Beijing strain was significantly more frequent among patients born abroad (13.6%, n = 3) than among patients born in Turkey (2.0%, n = 8; P = 0.016). The distributions of different spoligotypes according to birth place are shown in Table 3.

3.4. Infection site, microscopy positivity, drug sensitivity, and spoligotypes

Among the 421 strains studied, 268 (63.9%) were isolated from pulmonary TB and 131 (31.3%) from extrapulmonary TB patients' clinical samples, while 20 (4.8%) patients had both pulmonary and extrapulmonary TB and the remaining two isolates were from patients with undefined TB locations. The total pulmonary infection rate was 68.7%. Among the extrapulmonary TB patients, 48 had TB lymphadenitis, 27 pleural TB, 19 bone/joint/vertebra TB, eight TB peritonitis, eight skin and subdermal tissue TB, six TB meningitis, four urinary TB, and four TB pericarditis. The lowest pulmonary infection rates were observed in types ST52, ST47, and ST53 and the highest pulmonary infection rates in types ST1, ST284, and ST50 (Table 5). The lowest pulmonary infection rates were observed in families T (65.6%) and CAS (50%) and the highest pulmonary infection rates in families Haarlem (71.6%), Beijing (72.7%), and LAM (75.9%) and the undefined strains (79.6%).

The most predominant type, ST53, was detected in 36.2% of extrapulmonary TB and 18.1% of pulmonary TB among females, and in 18% of extrapulmonary TB and 24.9% of pulmonary TB among males. In contrast, the second predominant type, ST41, was detected in 8.7% of extrapulmonary TB and 4.8% of pulmonary TB among females, and in 4.9% of extrapulmonary TB and 8.3% of pulmonary TB among males.

Acid-fast bacilli (AFB) positivity was found in 50% (144/288) of pulmonary TB cases and 16.8% (22/131) of extrapulmonary cases. The lowest AFB positivity rates among pulmonary TB cases were detected in the ST41 and ST50 spoligotypes (35%), and the highest in ST1 (75%) and ST52 (83.3%). DST was applied for 396 patients and 14 strains showed resistance to RIF, with high-level resistance to INH in 8, low-level resistance to INH in 20, and multidrug resistance (MDR) in 12, of which six were type ST1. The MDR rate was 3% (Table 5).

Table 3. Comparison of mean ages and birth places of patients with specific types compared to the rest of the patients.

SIT number	Mean age (n = 421)			Birth place (n = 420)		
	Specified type	The rest of the patients	P	Aegean region	Other regions/abroad	P
53	54.3 ± 17.9	50.0 ± 20.1	0.043	61 (28.6)	44 (21.3)	0.081
41	45.9 ± 17.3	51.5 ± 19.8	0.137	12 (5.6)	17 (8.2)	0.297
50	49.3 ± 21.9	51.2 ± 19.5	0.643	10 (4.7)	14 (6.8)	0.361
284	48.6 ± 19.2	51.2 ± 19.7	0.547	14 (6.6)	8 (3.9)	0.213
47	57.2 ± 15.7	50.8 ± 19.8	0.193	12 (5.6)	5 (2.4)	0.094
4	59.6 ± 17.8	50.8 ± 19.7	0.077	9 (4.2)	7 (3.4)	0.652
1	40.0 ± 24.7	51.4 ± 19.4	0.057	1 (0.5)	10 (4.8)	0.005
52	48.8 ± 19.9	51.2 ± 19.7	0.709	4 (1.9)	6 (2.9)	0.539**
Other	50.1 ± 20.4	51.9 ± 19.0*	0.371	90 (42.3)	96 (46.4)	0.395

*The total of the most common eight types, **Fisher's exact test.

Table 4. The sex and age group distribution of different *M. tuberculosis* spoligotypes, compared to the rest of the patients (n, %).

SIT number	Sex (n = 421)			Age groups (n = 415)**				
	Females	Males	P	15–44	45–64	≥65	P	P trend
53	43 (24.6)	62 (25.2)	0.883	30 (19.1)	42 (29.6)	32 (27.6)	0.086	0.085
41	11 (6.3)	18 (7.3)	0.680	16 (10.2)	9 (6.3)	4 (3.4)	0.090	0.029
50	8 (4.6)	16 (6.5)	0.399	9 (5.7)	10 (7.0)	5 (4.3)	0.645	0.671
284	12 (6.9)	10 (4.1)	0.205	9 (5.7)	8 (5.6)	5 (4.3)	0.854	0.620
47	7 (4.0)	10 (4.1)	0.973	5 (3.2)	6 (4.2)	6 (5.2)	0.712	0.410
4	8 (4.6)	8 (3.3)	0.485	2 (1.3)	6 (4.2)	8 (6.9)	0.056	0.016
1	6 (3.4)	5 (2.0)	0.537*	8 (5.1)	0 (0.0)	3 (2.6)	NA	NA
52	4 (2.3)	6 (2.4)	1.000*	3 (1.9)	5 (3.5)	2 (1.7)	NA	NA
Other	76 (43.4)	111 (45.1)	0.730	75 (47.8)	56 (39.4)	51 (44.0)	0.349	0.460

*Fisher's exact test, **five patients <15 years old and one patient of unknown age were excluded.

Table 5. Site of infection, microscopy positivity, and drug resistance among the most common spoligotypes (n = 421).

SIT number	Infected organ (n = 419)		AFB positivity (n = 417)		MDR % (n/no. of strains with DST)
	Pulmonary* %	Extrapulmonary %	Positive	Negative	
53	69 (65.7)	36 (34.3)	36 (34.3)	69 (65.7)	0.0 (0/96)
41	20 (69.0)	9 (31.0)	8 (27.6)	21 (72.4)	3.6 (1/28)
50	20 (83.3)	4 (16.7)	7 (29.2)	17 (70.8)	0.0 (0/24)
284	18 (81.8)	4 (18.2)	11 (50.0)	11 (50.0)	0.0 (0/20)
47	10 (62.5)	6 (37.5)	8 (47.1)	9 (52.9)	0.0 (0/14)
4	12 (70.6)	5 (29.4)	6 (37.5)	10 (62.5)	0.0 (0/16)
1	8 (72.7)	3 (27.3)	7 (63.6)	4 (36.4)	54.5 (6/11)
52	6 (60.0)	4 (40.0)	7 (70.0)	3 (30.0)	10.0 (1/10)
Other	125 (67.6)	60 (32.4)	76 (41.5)	107 (58.5)	2.6 (4/177)
Total	288 (68.7)	131 (31.3)	166 (39.8)	251 (60.2)	3.0 (12/396)

*Patients having both pulmonary and extrapulmonary TB are classified as pulmonary TB cases.

4. Discussion

In Turkey, 59.5% of the new TB cases notified in 2010 were male and 40.5% were female patients (2), similar to our study. Worldwide, TB is known to be more common among males. Similar to the male-to-female ratio of 1.7 in European Union countries, 1.9 in other European countries, 1.7 in high-incidence countries, 1.7 worldwide, and 1.4 in Turkey, the male-to-female ratio of our cases was 1.4 (1).

In our study, the 27.6% proportion of the age group ≥65 years was higher than the rates of 13.7% in Turkey and 17.1% in İzmir, while the rate of the age group of 15–44 years (37.5%) was lower than its rate of 53.6% in Turkey and 43.5% in İzmir (2). The high older-age ratio could be linked to the tertiary care given at this university hospital. The rate of women aged 15–44 years was higher than men, while the rate of men aged 45–64 years was higher than women. Similarly, 41.2% of female patients and 36.5% of male patients were in the 15–34 age group in Turkey in 2010 and the 45–64 age group ratio was higher among males (30.6%) than females (24.6%). However, no explanation can be found for TB to be relatively more common among young women and among men aged 45–64 years (2).

In our study, the most predominant spoligotypes isolated among the 470 strains in 1996–2014 were ST53 with 24.7% and ST41 with 8.1%, followed by ST50, ST284, ST4, ST47, and ST1, respectively. Several studies in Turkey have found similar results, finding ST53 the most prevalent type with rates differing between 27.2% and 16.1%, followed by ST41 (8–11). However, in three different studies on strains isolated in Ankara and East Anatolia, ST41 was found as the most prevalent type with rates between 21% and 47.5%, followed by ST53 (12–14). The unexpectedly marked difference between the rates of ST53, ST41, and ST284 in two distinct studies from Ankara may be due to the study by Kisa et al. (11) that may represent Turkey more reliably because of military recruitment obligations. However, the marked difference in the ST41 ratios of two studies from East Anatolia, namely those of Otlu et al. (10) and Durmaz et al. (14), cannot be explained (Figure). In our study, similar to other studies from Turkey, we have found that the most dominant types were ST53 and ST41, followed by ST50 and ST284, and that ST1 is currently a rare strain in our country.

Although different studies on multidrug-resistant TB isolates in Turkey have found variations in the rate of the Beijing strains between 1.3% and 50% due to differences in their patient populations, all the studies have found very high MDR ratios, varying from 41.3% to 66.7% among the Beijing strains (15–17). In a study on 4069 *M. tuberculosis* strains isolated in İstanbul in 2002–2005, the prevalence of the Beijing genotype was 1.1% (46/4069), the MDR rate was 12.5% (510/4069), the Beijing genotype's prevalence among multidrug-resistant TB isolates was 3.7% (19/510), MDR was found in 41.3% (19/49) of the Beijing strains, and 10 out of the 19 Beijing strains were isolated from patients who were citizens of former Soviet Union countries (17). In three different studies conducted on drug-resistant and multidrug-resistant TB isolates in Turkey, the most common type was ST41 with rates of 26%, 22.5%, and 16.8%, and ST53 was the second most common with rates of 14%, 19.5%, and 15.8% (15,16,18). In our study, the rate of MDR was 3%, six of the 12 multidrug-resistant

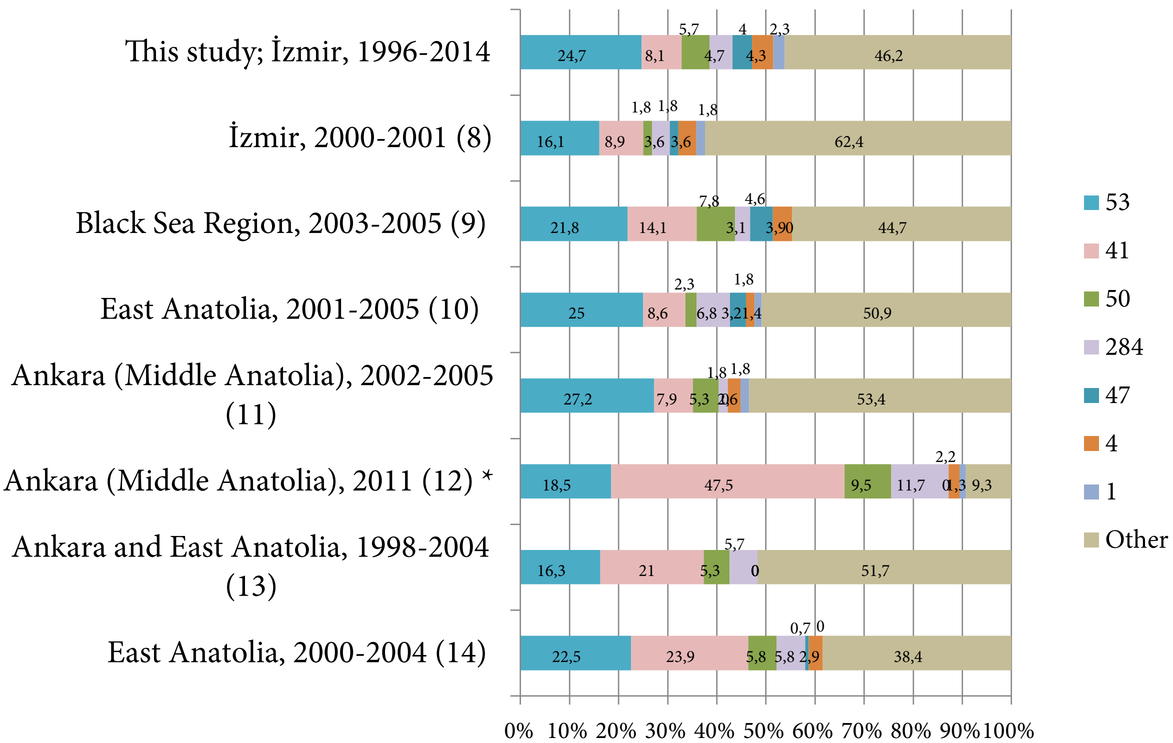

Figure. The distribution of spoligotypes in different studies on *M. tuberculosis* strains in Turkey: locations and years of the studies (%).
*P < 0.001 as compared to this study. Comparisons with all the other studies (8–11,13,14) were not valid due to expected counts of less than 5.

TB isolates being of the Beijing type and one being ST41. There was no ST53 among the multidrug-resistant TB isolates in our study. Of the Beijing strains, 54.5% (6/11) were multidrug-resistant and two of the six Beijing strains were isolated from former Soviet Union citizens. These findings indicate that drug resistance is less common in ST53, which is the dominant spoligotype in our area.

According to SITVITWEB (19), we may infer that ST53 and ST50 are common types worldwide as well, and ST41 and ST284, which are not commonly found worldwide, may be local strains encountered mostly in Anatolia. *M. tuberculosis* lineages show a strong phylogeographical population structure, with different lineages associated with distinct geographical regions. Lineages 1 and 3 show a more restricted geographical distribution limited to East Africa, India, and the shores of the Indian Ocean. The strains of lineage 1 and lineage 3 include EAI and CAS spoligotype clusters, respectively, and the EAI spoligotype cluster is accepted as containing the "ancient" *M. tuberculosis* strains (20). In this study, no isolate belonging to the EAI family was found and only 0.9% (4/470) of all isolates belonged to the CAS family. Lineage 2 includes the Beijing family of strains and is predominant in East Asia, but is also present in Central Asia and Russia. In this study, 2.3% (11/470) of all isolates were of the Beijing family and three of the 11 Beijing strains were isolated from former Soviet Union citizens. Lineage 4 includes "modern" spoligotype families such as T, Haarlem, LAM, and X. T is the most represented family (35%) in all of Europe and West Asia (35.4%). Haarlem is prevalent in East Europe and is the second most represented family in Europe (24%). The LAM family is also prevalent in South Europe (19). In our study, T was the most represented family (46.6%), followed by Haarlem (16%) and LAM (12.3%). The Anglo-Saxon ancestral X family was also very rare in our study (0.9%).

Infection site distribution of the samples obtained in 2009–2014 were similar to the distribution of cases reported in Turkey in 2010 with pulmonary TB at 61.1%, extrapulmonary TB at 35.1%, and both sites at 3.8% (2). Furthermore, the male-to-female ratio found for pulmonary TB as 2.13 and extrapulmonary TB as 0.75 in Turkey in 2010 is in concordance with our study's findings of 1.74 (183/105) in pulmonary and 0.89 (62/69) in extrapulmonary TB cases (2). In studies from different countries, extrapulmonary TB is found to have a higher ratio among women, and despite a few exceptions, the most common type of extrapulmonary TB encountered is peripheral lymphadenitis; this form is more prevalent among women than men (21–25). The most common extrapulmonary TB forms were peripheral lymphadenitis observed in 44.9% of women and 27.9% of men and pleural TB found in 15.9% of women and 26.2% of men, and we have concluded that the higher ratio of extrapulmonary TB among women is due to the higher prevalence of peripheral lymphadenitis among women. Different studies have found widely varying age groups with the highest extrapulmonary TB prevalence (21–24). In our study, similar to Turkey overall, the age group of 45–64 years showed the lowest prevalence of extrapulmonary TB among men (0.26%) in contrast to the highest extrapulmonary involvement in women (1.42%).

The clustering ratio in this study was lower for extrapulmonary TB (75.3%) than pulmonary TB (77.6%) and the dominant spoligotypes ST53 and ST41 were more prevalent among female extrapulmonary TB cases while they were more prevalent in male pulmonary TB cases. Similarly, Gonzales et al. (21) found a lower clustering ratio in extrapulmonary TB than pulmonary TB (65%). Although the dominant *M. tuberculosis* spoligotypes show wide variation throughout different regions of the world, the ratio of extrapulmonary TB, especially TB lymphadenitis, is globally higher among women than men (21,22). Although the reason for this is not clarified, links to cellular immunity, hormones, and transmission dynamics are postulated (23). The pulmonary infection rates of Haarlem, Beijing, and LAM strains, with rates of 71.6%, 72.7%, and 75.9%, were in concordance with the findings of Lari et al. (26). These rates were respectively higher than the average infection rate (68.7%), while we found the pulmonary infection rate of the T family (65.6%) to be lower than the average. Although the number of strains is very small, similar to Lari et al. (26) we also found an association between lineage 3 (CAS) strains and extrapulmonary disease with a rate of 50%.

Spoligotyping is the simplest technique for genotyping of *M. tuberculosis* complex strains. Although spoligotyping can be a remarkable method for molecular epidemiology studies of *M. tuberculosis*, its discriminatory power is inferior to that afforded by MIRU-VNTR and whole-genome analyses. It also cannot recognize mixed infections and is less informative in regions with predominant or endemic strains (27,28).

We detected a shift in the ratios of several spoligotypes throughout the years, with decreases observed in the ratios of ST41 and ST4 and increases observed for ST284 and ST1. Significant differences were also observed for infection sites and AFB positivity. Pulmonary infection and AFB positivity were higher than average in Beijing and ST284 (one of the local, country-specific strains), while they were lower in the ST41 spoligotype. As pulmonary TB and AFB positivity can be considered as factors increasing infectivity, with increasing migration from less developed countries and from the former Soviet

Union to Turkey, increase in the number of HIV-infected and other immunocompromised hosts, and its increasing trend throughout the years, the Beijing strain, with its high MDR rate, could pose a potential threat to the tuberculosis control program in our region. Similarly, with its higher pulmonary involvement and AFB positivity rate, ST284 could also replace ST41 in the following years in this region with its increasing trend.

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